camera, and the adaptation of the phase-ramp 3D method to encode fluorophore color in the relative intensity of the two PSF lobes.

2394-Pos Board B531

Three-Dimensional Super-Resolution Protein Localization Correlated with Vitrified Cellular Context

Wei Ji, Bei Liu, Yanhong Xue, Wei Zhao, Tao Xu.
Institute of Biophysics,CAS, Beijing, China.

We demonstrate a cryogenic super-resolution correlative light and electron microscopy of vitrified specimens which were prepared by high pressure freezing and cryo-sectioning to maintain a close-to-native state with better fluorescence preservation. Several fluorescent proteins were found photo-switchable and emitted much more photons under cryo-condition, hence resulting in higher localization precision. We observed nice correlation of a mitochondria protein with mitochondria outer membrane at nanometer resolution in three dimensional.

2395-Pos Board B532

3D Microscopy of Rod-Shaped Bacteria Reveals Roles of MreB in Diameter Control and Center-Line Curvature

Benjamin P. Bratton[1,2], Jeffrey P. Nguyen[3], Nikolay Ouzounov[1], Randy M. Morgenstein[1], Zemer Gitai[1], Joshua P. Shaevitz[2,3].
[1]Department of Molecular Biology, Princeton University, Princeton, NJ, USA, [2]Lewis-Sigler Institute for Integrative Genomics, Princeton University, Princeton, NJ, USA, [3]Department of Physics, Princeton University, Princeton, NJ, USA.

Over the past few years, our lab has developed a method for precisely determining the shape of bacterial cells in 3D by fluorescence microscopy. We use this method to measure the position of MreB in relation to the cell surface. To ensure that our measurements of fluorescent MreB reflect untagged MreB as nearly as possible, we have integrated our fusion at the native locus. This construct has unperturbed mass doubling times and proper rod-like shape. The shape and localization measurements that we report here are measured as snapshots from hundreds to a few thousand cells per condition.

MreB, a membrane-binding structural homolog of actin, is one of the key players in properly patterning growth of the bacterial cell wall and thereby the shape of the cell. In one series of experiments, we show that the helical pitch of MreB filaments in E. coli is highly correlated to the diameter of the cell. This correlation holds for E. coli whose diameter has been altered by treatment with sub-lethal doses of the MreB targeting drug A22 and for single point mutants in MreB.

Additionally, MreB polymers show a clear preference for regions of negative Gaussian curvature. We hypothesize that cells use this geometric sensing mechanism to straighten their centerlines [Ursell et al., PNAS 2014]. When this curvature preference is abolished (E. coli MreB A158T), cells are more frequently branched. In the smaller, comma shaped bacterium Caulobacter crescentus, the curvature enrichment profile shows a plateau region at low positive Gaussian curvature. This plateau enables Caulobacter cells to grow with their characteristic comma shape instead of as straight rods.

2396-Pos Board B533

Enabling Single-Molecule Detection in Living Cells: Ultra-Sensitive Microscopy and Spectroscopy in 3D

Ankun Dong[1,2], Alexandros Pertsinidis[1].
[1]Molecular Biology, MSKCC, New York, NY, USA, [2]Brown University, Providence, RI, USA.

Single-molecule approaches can now allow us to follow the movement, interactions and conformational dynamics of individual molecules in real-time, thus providing novel insights in complex biochemical systems that have remained masked in the ensemble averaging of traditional bulk biochemical approaches. However, most single-molecule experiments are based on in vitro reconstituted systems with either surface-immobilized or freely-diffusing biomolecules in dilute conditions. Observing individual biomolecules in their native, crowded intracellular environment currently remains an extremely challenging task.

Pushing the limits of spatial/temporal resolution, and more importantly detection sensitivity, to enable performing single-molecule assays using fluorescently labeled biomolecules inside living cells would open many new frontiers in biochemical investigations; however few generally applicable viable approaches have been described. Here we show the theoretical basis and experimental validation of a new approach based on spatially-targeted manipulation of the fluorescence capabilities of single individual fluorophores. We show that with appropriately shaped on-off switching light-fields as well as by carefully considering the time-evolution of the fluorescent state at different xyz positions, sharper single-molecule images can be obtained. Furthermore, we demonstrate increased sensitivity in real-time detection of inter-molecular associations of individual target-probe molecular partners. Our results additionally indicate the potential for increased sensitivity using our approach to detect individual intracellular factors, such as the components of the RNA Polymerase II transcription machinery in the nucleus of single-living cells.

2397-Pos Board B534

Analysis of Nanoscale Protein Clustering with Quantitative Localization Microscopy

Philip R. Nicovich[1], Thibault Tabarin[2], Johannes Stiegler[2], Sophie V. Pageon[2], Katharina Gaus[1].
[1]Centre for Single Molecule Science, University of New South Wales, Sydney, Australia, [2]Centre for Vascular Research, University of New South Wales, Sydney, Australia.

Dynamic clustering of proteins on the nanoscale is a vital step in many signaling processes and other cellular functions. Localization microscopy techniques such as PALM and dSTORM provide methods to localize complexes to nanoscale resolution. Quantification of the number of underlying protein subunits is more difficult, however, due to the complex photophysics of the fluorophore labels. Re-activation, stochastic blinking, and incomplete detection all contribute to over- and under-counting artifacts.

In order to generate an accurate quantification of protein subunit numbers we have developed a method based on fluorophore blinking kinetics captured in PALM microscopy. This approach takes advantage of both spatial and temporal information to form adaptive discrimination criteria and avoids both over- and under-counting quantification errors. The technique presented can correctly identify and quantify molecular clusters in simulated data with 98% accuracy. We will present additional data on the application of this technique in quantifying the nature of clusters of proteins involved in the immune synapse and immune signaling.

2398-Pos Board B535

Video-Rate Super Resolution Microscopy in Living Cells

Fang Huang[1], Caroline E. Laplante[2], Yu Lin[1], Thomas D. Pollard[2], Joerg Bewersdorf[1].
[1]Department of Cell Biology, Yale University, New Haven, CT, USA, [2]Department of Molecular, Cellular and Developmental Biology, Yale University, New Haven, CT, USA.

Super resolution microscopy based on single-molecule localization relies on precise and accurate localization of large numbers of single-molecules. However, the necessity of accumulating large numbers of localization estimates limits the time resolution typically to seconds to minutes[1,2].

Two major limitations are the acquisition speed and the photon budget. Replacing the usually used EMCCD with a recently introduced sCMOS camera results in leaps in both acquisition speed and effective quantum efficiency. However, the intrinsic pixel-dependent Gaussian noise of the sCMOS cameras introduces localization artifacts and greatly reduces the reliability of the results.

Here, we present a set of specially designed methods that characterize an sCMOS camera for the first time and allow for unbiased and precise localization analysis. Using this method we demonstrate Cramer-Rao lower bound-limited single-molecule localization with an sCMOS camera. Combining the novel algorithm with a recently developed multi-emitter fitting algorithm[3], We shortens the typical acquisition time for fixed samples by up to two orders of magnitude without compromising the field of view. Furthermore, we demonstrate localization-based super-resolution microscopy in live cells by monitoring dynamics of protein clusters, vesicles and organelles at a temporal resolution from 2 to 30 frames per second[4].

These methods allowed us to investigate cytokinetic apparatus in live fission yeast at 20-30 nm resolution. In general, the significantly improved temporal resolution allows super resolution imaging of a large range of dynamic events in living cells.

1. Patterson, G., Davidson, M., Manley, S. & Lippincott-Schwartz, J. *Annu. Rev. Phys. Chem.***61,** 345-67 (2010).
2. Gould, T. J., Hess, S. T. & Bewersdorf, J. *Annu. Rev. Biomed. Eng.***14,** 231-54 (2012).
3. Huang, F., Schwartz, S. L., Byars, J. M. & Lidke, K. A. *Biomed. Opt. Express* **2,** 1377-93 (2011).
4. Huang, F. *et al.Nat. Methods* **10,** 653-8 (2013).

2399-Pos Board B536

Dual-Objective Pointillism Microscopy Setup with Interferometric and Astigmatic Detection

Nora C. Schmidt[1], Jana Hüve[1,2], Jürgen Klingauf[1].
[1]Institute of Medical Physics and Biophysics, Münster, Germany, [2]Fluorescence Microscopy Facility Münster, Münster, Germany.

In recent years, under the general term of pointillism microscopy many techniques have been developed which employ sequential imaging of photoswitching fluorophores to circumvent the diffraction barrier in light microscopy